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Award Number: W81XWH-11-1-0405

TITLE: Induced pluripotent stem cell derived mesenchymal stem cells for attenuating age-related bone loss

PRINCIPAL INVESTIGATOR: Henry J. Donahue, Ph.D.

CONTRACTING ORGANIZATION: Pennsylvania State University, Milton S. Hershey Medical Center
Hershey, PA 17033-0850

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14. ABSTRACT

Osteoporosis, both age-related and post-menopausal, is a huge health problem in the United States and indeed worldwide. Despite extensive research there remain few therapeutic approaches, with the exception of parathyroid hormone, that actually increase bone formation in osteoporotic patients. There are several limitations to the use of parathyroid hormone suggesting the need for continued research into anabolic therapies for osteoporosis.1 Mesenchymal stem cell (MSC) differentiation towards the bone forming osteoblastic lineage decreases as a function of age and may contribute to age-related bone loss.2 Therefore, MSC therapy may be beneficial in treating age-related bone loss. However, MSC availability decreases with age.2 To overcome the problem of age-related reduced availability of MSC we propose to examine the bone anabolic potential of induced pluripotent stem cell (iPS) derived MSC in age-related bone loss. Unfortunately deriving MSC from iPS can require extended in vitro culture, which decreases the differentiation potential of MSC.3 Since biomaterial surface characteristics, including stiffness and topography, can control MSC differentiation in vitro, including toward the osteoblastic lineage,4,5 the goal of this project is to identify biomaterial surface characteristics that enhance differentiation of iPS toward MSC and MSC toward osteoblastic cells. Our hypothesis is that culturing iPS on nanotopographic surfaces results in enrichment of a population of cells exhibiting MSC characteristics. Continued culture of these iPS derived MSC on nanotopographies results in increased osteoblastic differentiation and increased potential to induce bone formation in senescent accelerated mice (SAMP6), a murine model of age-induced bone loss. Successful completion of the specific aims would suggest a novel and highly innovative therapeutic approach to age-related osteoporosis.

15. SUBJECT TERMS- none provided

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Annual Report for Award Number W81XWH-11-1-0405

Induced pluripotent stem cell derived mesenchymal stem cells for attenuating age-related bone loss

Covering July 1, 2011 through June 30, 2012

Principal Investigator: Henry J. Donahue, Ph.D.

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Introduction

Osteoporosis, both age-related and post-menopausal, is a huge health problem in the United States and indeed worldwide. Despite extensive research there remain few therapeutic approaches, with the exception of parathyroid hormone, that actually increase bone formation in osteoporotic patients. There are several limitations to the use of parathyroid hormone suggesting the need for continued research into anabolic therapies for osteoporosis.[1] Mesenchymal stem cell (MSC) differentiation towards the bone forming osteoblastic lineage decreases as a function of age and may contribute to age-related bone loss.[2] Therefore, MSC therapy may be beneficial in treating age-related bone loss. However, MSC availability decreases with age.[2] To overcome the problem of age-related reduced availability of MSC we propose to examine the bone anabolic potential of induced pluripotent stem cell (iPS) derived MSC in age-related bone loss. Unfortunately deriving MSC from iPS can require extended in vitro culture, which decreases the differentiation potential of MSC.[3] Since biomaterial surface characteristics, including stiffness and topography, can control MSC differentiation in vitro, including toward the osteoblastic lineage,[4,5] the goal of this project is to identify biomaterial surface characteristics that enhance differentiation of iPS toward MSC and MSC toward osteoblastic cells. Our hypothesis is that culturing iPS on nanotopographic surfaces results in enrichment of a population of cells exhibiting MSC characteristics. Continued culture of these iPS derived MSC on nanotopographies results in increased osteoblastic differentiation and increased potential to induce bone formation in senescent accelerated mice (SAMP6), a murine model of age-induced bone loss. Successful completion of the specific aims would suggest a novel and highly innovative therapeutic approach to age-related osteoporosis.

Body

On July 27, 2012 we requested a 6 month extension without funding for this project. This extension was approved December 12, 2012 (see attached). Therefore, this report represents progress through July 30, 2012.

During the first year of this project we made considerable progress towards the completion of tasks 1 and 2. However, we were unable to get to a point where we actually injected iPSC to osteopenic mice (task 3). This is due to the fact that the time it took to develop iPS was longer than initially anticipated. However, the progress we made in establishing iPS[6] and in demonstrating that polymer nanotopographies enhanced hMSC osteoblastic differentiation[7] warrant continued examination of the potential of polymer nanotopographies to enhance iPS osteoblastic differentiation. The following outlines our results as regards each task.

Task 1. Examine the hypothesis that specific nanoscale topographies will increase differentiation of iPS toward the MSC lineage. We will examine expression of MSC markers on iPS cultured on specific nanoscale topographies.

We were able to demonstrate that we could generate iPS that differentiated into mesenchymal stem cells. This is a critical first step in examining whether polymer nanotopographies affect iPS differentiation towards mesenchymal stem cells. Differentiation of the cells derived from iPSC using different treatments, were assessed for differentiation into osteoblast in vitro. Cells were cultured in vitro in a medium supplemented with osteogenic supplements. At 21 days following cultivation, cells were assessed for mineral deposition. Results indicated that cells derived by different treatments differentiated into osteoblasts and deposited bone in vitro but with varying degrees. TGF-b1 treated cells showed most mineral deposition. Interestingly, BMP-2 treated cells exhibited a higher potential to differentiate toward osteoblasts.

Differentiation of the cells toward osteogenic and adipogenic lineage are shown in figure 1. Quantification of mineral deposition by the iPS cells was also evident. A manuscript describing these results is currently in preparation. We are now in an excellent position to examine the effect of polymer nanotopographies on iPS differentiation to MSC.

Task 2. Examine the hypothesis that specific nanoscale topographies increase the differentiation of MSC identified in aim 1 toward the osteoblastic lineage. We will examine expression of osteoblastic phenotypic markers on iPS derived MSC cultured on nanotopographic biofilms.

While we have not yet examined the effect of polymer nanotopographies on iPS differentiation to MSC, we have demonstrated that polymer nanotopographies enhance hMSC differentiation towards the osteoblastic lineage. We found that culture on specific scale (10-20 nm high) polymer nanoislands accelerates hMSC lineage commitment toward osteogenesis, as seen by increased alkaline phosphatase (AP) activity and mineralization (Fig. 2;[7]). We further observed that the accelerated differentiation of hMSCs on specific nanotopographies could be detected in the form of decreased stem cell surface markers (Fig. 3;[7]). That hMSCs lose stem cell surface markers (SSEA-4, CD73, CD105) on 11 nm high nanoislands indicates that these cells are no longer stem cells (or already committed to a specific fate, in this case osteogenesis). These data suggest that specific nanoscale topography potentiates hMSC differentiation toward the osteoblastic lineage and therefore has the potential to induce iPSC differentiation toward MSC. These results have been published in the Journal of Orthopaedic Research.

Task 3. Examine the hypothesis that MSC, derived from iPS, cultured on nanotopographies enhance bone formation, in a murine model of aged-induced bone loss, to a greater degree than MSC derived from iPS cultured on flat surfaces.

We have found that culturing iPS cells on nanotopographies to be more challenging than originally envisioned. Therefore we requested and were granted additional time but no additional funds to attempt to complete a revised statement of work. We have been successful in generating iPS cells and will continue to develop protocols to culture these cells on nanotopographies. Normally iPS cells once generated are expanded on gelatin. We have had difficulty incorporating the gelatin coating on nanotopographies but believe we now have a solution to the problem. Unfortunately due to unanticipated problems expanding iPS cells on nanotopographies and the limited duration of the project, it is no longer feasible to attempt the in vivo studies we had planned. It is our hope to complete tasks 1 and 2 in the next six months. If we are successful, we will seek funding to continue with in vivo studies.

Key Research Accomplishments

- Demonstrated the ability to generate iPSC that differentiate into MSC that form bone in vivo.
- Demonstrated that polymer nanotopographies enhance osteoblastic differention of hMSC and therefore have the potential to do so for iPS.

Reportable Outcomes

Lim, J.Y., Loiselle, A.E., Lee,J.S., Zhang, Y., Salvi, J. D., and **Donahue, H.J.** (2011) Optimizing the osteogenic potential of adult stem cells for skeletal regeneration. J. Orthopaedic Research, 29(11):1627-33.

Li, Feng Niyibizi, Christopher. Induction of induced Pluripotent Stem cells (iPSC) to Multotent Mesenchymal Stem Cells and in vivo Bone Formation. 58th Annual Meeting of the Orthopeadic Research Society, San Francisco CA, Feb 2012.

Conclusion

Our results suggest that we can indeed induce pluripotent stem cells to differentiate into bone forming osteoblastic cells. Additionally, surface nanotopography affects hMSC to differentiate to osteoblastic cells. During the remainder of the project we will examine whether surface nanotopography affects differentiation of iPSC-induced MSC to differentiate to osteoblastic cells.

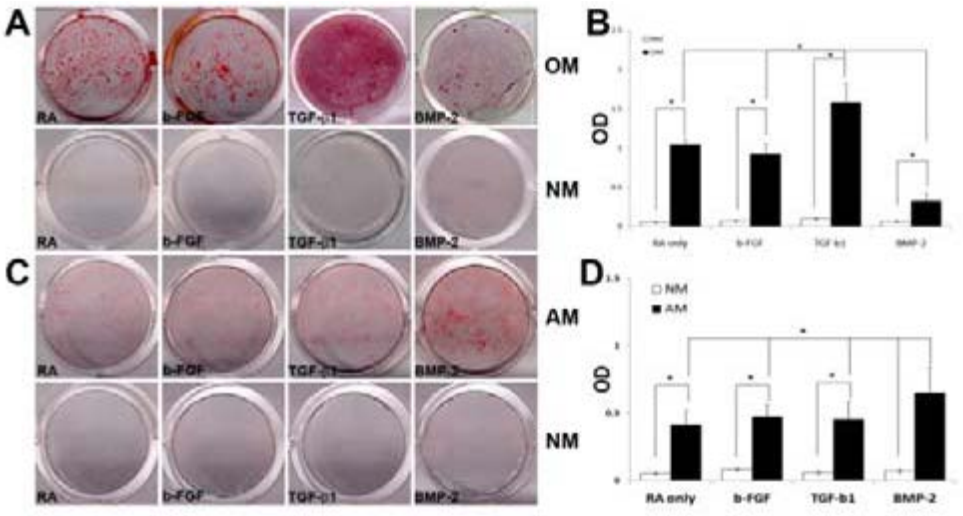

Figure 1.. Bone formation in vivo by iPSC TGF-derived MSCs with or without pre-BMP-2 treatment. A) pre-BMP-2treatment; B) No pre BMP-2 treatment. Pre-BMP-2 treatment enhances bone formation.

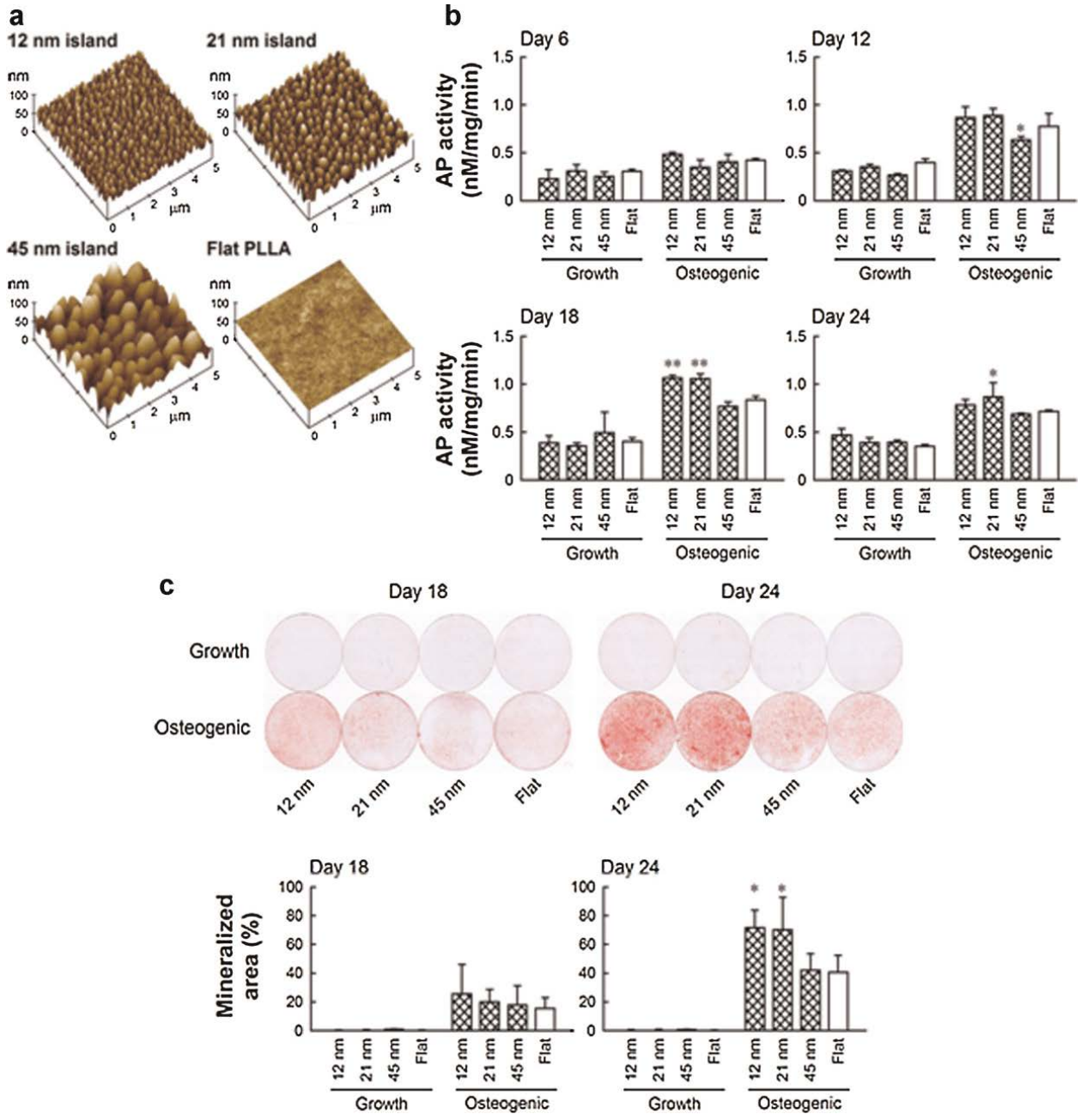

Figure 2. hMSC differentiation toward the osteoblastic lineage is enhanced when cells are cultured on specific nanoscale topographies. (a) Nanoisland topographies with varying island heights (12, 21, and 45 nm) produced by PLLA/PS (70/30 w/w) demixing. AFM height images of nanoislands and flat control surfaces are shown. (b) hMSCs were cultured on test surfaces in growth or osteogenic differentiation media and AP activity quantified (n=4). (c) Mineralized area. (n= 6).$p < 0.05$,$p < 0.01$ compared with flat PLLA; ANOVA with post hoc test.

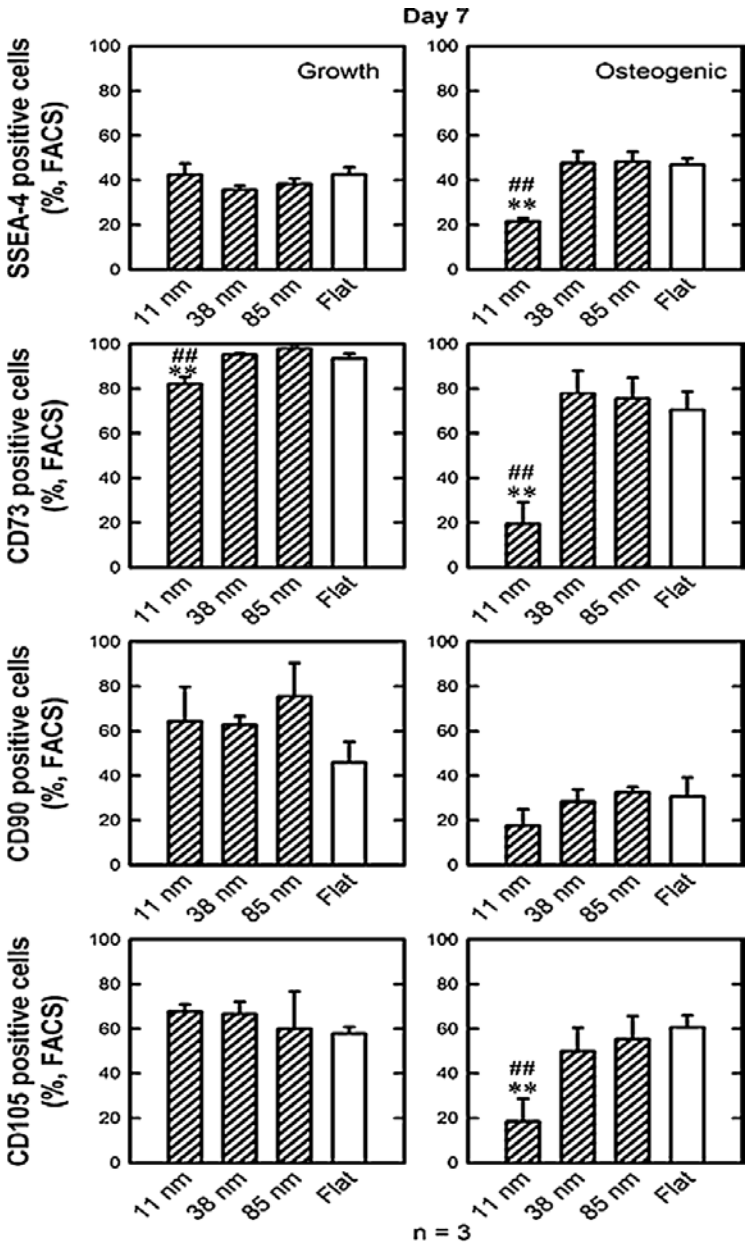

Figure 3. Stem cell surface marker expression in hMSCs on nanotopographies and flat control. Human MSCs were cultured on PS/PBrS films with growth or osteogenic media for 7 days, harvested, and tagged with antibodies for SSEA-4, CD73, CD90, and CD105. Flow cytometry was then utilized to determine the percent of the cell population with a positive stem cell surface marker. ##$p < 0.01$ compared among nanoislands; **$p < 0.01$ compared with flat control. ANOVA with post hoc test.

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3. Banfi A, Muraglia A, Dozin B, Mastrogiacomo M, Cancedda R, Quarto R. Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy. Exp Hematol 2000;28:707-15. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10880757

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6. Li, F. Niyibizi, C. Induction of induced Pluripotent Stem cells (iPSC) to Multotent Mesenchymal Stem Cells and in vivo Bone Formation. In: 58th Annual Meeting of the Orthopeadic Research Society San Francisco, Ca.; 2012.

7. Lim JY, Loiselle AE, Lee JS, Zhang Y, Salvi JD, Donahue HJ. Optimizing the osteogenic potential of adult stem cells for skeletal regeneration. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 2011. http://www.ncbi.nlm.nih.gov/pubmed/21509820

Appendices

See attached

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1. CONTRACT ID CODE	PAGE OF PAGES
S	1 | 3

2. AMENDMENT/MODIFICATION NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQ. NO.	5. PROJECT NO.(If applicable)
P00001	04-Jan-2013	W91ZSQ1022N640	

6. ISSUED BY	CODE	7. ADMINISTERED BY (If other than item 6)	CODE
USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014	W81XWH	US ARMY MEDICAL RESEARCH ACQUISITION ACT ATTN: MIRLENE ELLISON 301-619-7733 820 CHANDLER STREET FORT DETRICK MD 21702	W81XWH

8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code)		
PENNSYLVANIA STATE UNIVERSITY, THE MICHAEL S. YARNELL 500 UNIVERSITY DR C1607 HERSHEY PA 17033-2360		9A. AMENDMENT OF SOLICITATION NO.
		9B. DATED (SEE ITEM 11)
	X	10A. MOD. OF CONTRACT/ORDER NO. W81XWH-11-1-0405
	X	10B. DATED (SEE ITEM 13) 01-Jul-2011

CODE 7W765 FACILITY CODE

11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS

☐ The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer ☐ is extended, ☐ is not extended.

Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.

12. ACCOUNTING AND APPROPRIATION DATA (If required)

13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS. IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.

☐ A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.

☐ B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).

☐ C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:

X D. OTHER (Specify type of modification and authority)
IAW USAMRAA FDP Specific Requirements.

E. IMPORTANT: Contractor ☒ is not, ☐ is required to sign this document and return _____ copies to the issuing office.

14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.)

Modification Control Number: mellison131436

The purpose of this modification to:

1. Extend the period of performance end date from 30 July 2012 to 30 July 2013 (Research Ends 30 June 2013) at no cost to the government in accordance with the recipient request dated 27 July 2012; and GOR approval of the 6 month EWOF dated 12 December 2012
2. An annual Technical report will be due no later than 30 July 2012
3. The final technical report will be due no later than 30 July 2013.
4. Quarterly reports will continue to be provided.
5. All other terms and conditions remain unchanged.

Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.

15A. NAME AND TITLE OF SIGNER (Type or print)		16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) SUSAN DELLINGER / CONTRACTING OFFICER TEL: 301-619-2090 EMAIL: susan.dellinger@amedd.army.mil	
15B. CONTRACTOR/OFFEROR _____ (Signature of person authorized to sign)	15C. DATE SIGNED	16B. UNITED STATES OF AMERICA BY Susan M. Dellinger (Signature of Contracting Officer)	16C. DATE SIGNED 04-Jan-2013

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

CLIN 0001

The CLIN extended description has changed from Peer Reviewed Medical Research Program (PRMRP)- Concept AwardPeriod of Performance: 1 July 2011 - 30 July 2012 (research ends 30 June 2012) to Peer Reviewed Medical Research Program (PRMRP)- Concept AwardPeriod of Performance: 1 July 2011 - 30 July 2012 (research ends 30 June 2012) to 30 July 2013 (research ends 30 June 2013).

DELIVERIES AND PERFORMANCE

The following Delivery Schedule item for CLIN 0001 has been changed from:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
POP 01-JUL-2011 TO 30-JUL-2012	N/A	USA MED RESEARCH MAT CMD 1077 PATCHEL STREET BLDG 1077 FORT DETRICK MD 21702 FOB: Destination	W91ZSQ

To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
POP 01-JUL-2011 TO 30-JUL-2013	N/A	USA MED RESEARCH MAT CMD 1077 PATCHEL STREET BLDG 1077 FORT DETRICK MD 21702 FOB: Destination	W91ZSQ

SECTION 00800 - SPECIAL CONTRACT REQUIREMENTS

The following have been modified:

A. This award is made under the authority of 31 U.S.C. 6304 and 10 U.S.C. 2358. The recipient's statement of work and the budget for this proposal submitted in response to the Fiscal Year 2010(FY10) Department of Defense Peer Reviewed Medical Research Program (PRMRP) – Concept Ward Announcement, which closed April 8, 2010, are incorporated herein by reference. The Catalog of Federal Domestic Assistance Number relative to this award is CFDA 12.420.

(End of Summary of Changes)